Analysis of Genetic Diversity And DNA Fingerprinting In Early-Maturing Upland Cotton Using SSR Markers

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Abstract

In this study, DNA fingerprinting and genetic diversity analysis of 79 early-maturing upland cotton (*Gossypium hirsutum* L.) cultivars were performed using Simple Sequence Repeat (SSR) molecular markers. From 126 pairs of SSR primers, we selected 71 pairs of primers that gave good polymorphisms and clear bands, had good stability, and showed even distribution on the cotton chromosomes, and 142 polymorphic genotypes were amplified. The average number of alleles amplified with the SSR primers was 2.01. The polymorphism information content (PIC) of the markers ranged from 0.1841 to 0.9043 with an average of 0.6494. The results of fingerprint analysis showed that nine varieties had characteristic bands, and at least six primer pairs could be used to completely distinguish all 79 cotton accessions. Using NTSYS-pc 2.11 cluster analysis, the genetic similarity coefficients between the cotton genotypes ranged from 0.3310-0.8705, with an average of 0.5861. All cotton accessions were grouped into five categories at a similarity coefficient of 0.57, which was consistent with the pedigree sources. At the same time, the average genetic similarity coefficients of early-maturing upland cotton varieties in China showed a low-high-low pattern of variation over time, revealing the development history of early-maturing upland cotton varieties from the 1980s to the present. This also indirectly reflects that in recent years, China's cotton breeders have focused on innovation and have continuously broadened the genetic resources for early-maturing upland cotton.

Introduction

Cotton is an important economic crop in China and the leading raw material used in the textile industry¹⁻³. In recent years, cotton cultivation in China has shown a trend of moving eastward, westward, and northward⁴. In order to ensure safety of cotton production and utilization, China will continue to maintain the three existing cotton-producing regions; the Northwest inland (Xinjiang), the Yellow River Basin (YRB), and the Yangtze River Basin (YTRB) for the foreseeable future. However, expansion of cotton production in Xinjiang is limited by water shortages. The cotton plantation area in YTRB will be maintained at 660,000 hm² well into the future because of its suitable geographical climate, developed cotton spinning industry, and stable market demand. Cotton is the dominant crop in YTRB, although its competitiveness is weak due to its long growth period, high labor requirements, and high production cost. Therefore, it is urgent to select and breed new cotton varieties suitable for mechanized production in order to reduce labor costs and increase cotton planting efficiency⁵.

Early-maturing upland cotton has the typical characteristics of a relatively short growth period and concentrated flowering and boll opening⁶⁻⁷. Early-maturing upland cotton is one of the main targets of cotton breeding in YTRB for the future; this will not only allow for two crops per year by rotation with winter crops such as wheat and rape, but is also suitable for mechanized harvesting to achieve simple and efficient cotton production⁸. A total of 79 early-maturing cotton accessions were collected and introduced from northern China to improve the local core germplasm resources that have long growth periods. To fully realize the genetic variation present in the introduced germplasm resources, it is necessary to study the genetic diversity of the 79 accessions.

Molecular marker technology is one of the main tools used for studying the genetic diversity of cotton varieties both in China and overseas, and the marker types include but are not limited to restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs)⁹⁻¹¹. Of these marker

types, SSRs have the advantages of high polymorphism, good reproducibility, co-dominance, and simple operation¹²⁻¹³. SSRs have been used for cotton DNA fingerprinting, genetic diversity analysis, and QTL mapping^{8,14-18}, and this has enhanced the protection of cotton germplasm resources and enabled the genetic improvement of cotton varieties in China. In the current research, DNA fingerprinting, genetic diversity, and the genetic relationships among 79 early-maturing upland cotton accessions were analyzed with SSR markers. The results of our study will provide genetic resources for the breeding of new varieties of early-maturing upland cotton in Hunan and YTRB through a systematic understanding of the genetic backgrounds of 79 early-maturing upland cotton germplasm accessions.

1. Materials And Methods

1.1 Experiment material

A total of 79 early-maturing upland cotton germplasm obtained from the National Cotton Mid-term Gene Bank (Anyang, Henan, China) were used for this study. All materials were used with the National Cotton Mid-term Gene Bank's permission and national guidelines. The maturity information of these materials is also provided by the Gene Bank. These materials were collected by the National Cotton Mid-term Gene Bank from six regions, including the Institute of Cotton Research of Chinese Academy of Agricultural Sciences (ICR-CAAS) which we consider to be a separate branch because it is a national institute and its cotton varieties are *sui generis* (of their own kind), YRB (Henan, Shanxi, Shandong, Jiangsu), the Northwest Inland Region (Xinjiang, Gansu), the Liaohe River Basin, the United States, and the former Soviet Union (Table 1). All materials were planted in the Deshan Experimental Field of the Hunan Institute of Cotton Sciences Research in 2015. We investigated the maturity of these materials and all growth period of the materials were less than 110 days in Hunan province. Polymorphic SSR markers were selected from testing in other materials for many years, and the primers were synthesized by Shanghai Yingjun Biotechnology Co., Ltd. PCR reagents (Taq DNA polymerase, dNTPs, DNA marker size standard) were purchased from Beijing Qianjin Biotechnology Co., Ltd. Our studies did not involve any endangered or protected species.

Table 1. The names, certification years, and pedigrees of the 79 early-maturing cotton accessions

Number	Cultivars name	Years	Region	Pedigrees
1	ICR-CAAS10	1980	Anyang	Heishanmian1-derived Lines
2	ICR-CAAS14	1989	Anyang	211×Liao6913
3	ICR-CAAS16	1990	Anyang	Zhong 211×ICR-CAAS10-derived Lines×Liao4086×Liaomian7-derived Lines
4	ICR-CAAS18	1990	Anyang	(6908×Lambretta GL-5)F ₄ × ×Heishanmian1×Lambretta GL-5)F ₃
5	ICR-CAAS24	1995	Anyang	Zhong 343× (ICR-CAAS10×USA early maturity cotton B)
6	ICR-CAAS20	1996	Anyang	×Shan 2942×Zhong 642× F ₁ ×[×Zhong 607×Liao4086×PR80]F ₁
7	ICR-CAAS26	1996	Anyang	006×ICR-CAAS10
8	ICR-CAAS27	1997	Anyang	Zhong 0710×Zhong 5427
9	ICR-CAAS35	1999	Anyang	Zhong 23021×ICR-CAAS12×Chuan1704
10	ICR-CAAS36	1999	Anyang	H109×Zhong 662
11	ICR-CAAS37	1999	Anyang	Zhong R114-derived Lines
12	ICR-CAAS42	2002	Anyang	Zhong 061723×Zhong 916448
13	ICR-CAAS50	2005	Anyang	H109×C662
14	ICR-CAAS58	2006	Anyang	SGK 27×92-047
15	ICR-CAAS64	2007	Anyang	SGK 27×Zhong 394
16	Xinluzao1	1978	the Northwest Inland Region Xinjiang	Xin 722-derived Lines×69-1
17	Xinluzao3	1988	the Northwest Inland Region Xinjiang	×66-341×USA Glandless Cotton×Jingzhou 4588
18	Xinluzao4	1994	the Northwest Inland Region Xinjiang	×66-241×74-47W×DP 70
19	Xinluzao6	1997	the Northwest Inland Region Xinjiang	85-174×Bailschlo
20	Xinluzao7	1997	the Northwest Inland Region Xinjiang	347-2×Tashkent 2
21	Xinluzao9	1999	the Northwest Inland Region Xinjiang	(Xinluzao6×Bailschlo) ×ICR-CAAS17

22	Xinluzao10	1999	the Northwest Inland Region 新疆	(Heishanmian×02) ×Zhong 381
23	Xinluzao11	1999	the Northwest Inland Region 新疆	Yuzao 202-derived Lines
24	Xinluzao12	2000	the Northwest Inland Region 新疆	Liao 95-25-derived Lines
25	Jiumian2	2001	the Northwest Inland Region 甘肃	77-118×ICR-CAAS16
26	Xinluzao13	2002	the Northwest Inland Region 新疆	83-14×Zhong 5601+1639
27	Xinluzao20	2005	the Northwest Inland Region 新疆	Xinluzao16-derived Lines
28	Xinluzao22	2005	the Northwest Inland Region 新疆	45-1×Xinluzao6
29	Xinluzao23	2005	the Northwest Inland Region 新疆	ICR-CAAS27-derived Lines
30	Xinluzao25	2006	the Northwest Inland Region 新疆	[Xi 5×Bailschlo)×Jin14]F ₁ ×ICR-CAAS17
31	Xinluzao26	2006	the Northwest Inland Region 新疆	Xinluzao 8 Variant Plant
32	Xinluzao27	2006	the Northwest Inland Region 新疆	7147×Bailschlo
33	Xinluzao30	2006	the Northwest Inland Region 新疆	97-185-derived Lines
34	Xinluzao31	2006	the Northwest Inland Region 新疆	新疆luzao6×Bailschlo×Daizimian
35	Xinluzao32	2006	the Northwest Inland Region 新疆	Takla Makan 77-derived Lines
36	Xinluzao34	2007	the Northwest Inland Region 新疆	Shi 87 Variant Plant
37	Xinluzao35	2007	the Northwest Inland Region 新疆	新疆(Xinluzao3×Zhong 2621)×Kan 35×97-185

38	Xinluzao36	2007	the Northwest Inland Region 新疆	1304×BD103
39	Xinluzao37	2007	the Northwest Inland Region 新疆	Xi 8×(Liao 9001,Xi 5 and 90-2)
40	Jiumian3	2007	the Northwest Inland Region 甘肃	Liaomian16 Variant Plant
41	Xinluzao39	2008	the Northwest Inland Region 新疆	Xinluzao4×Bailschlo
42	Jiumian8	2008	the Northwest Inland Region 甘肃	Zhong 404×Xinluzao7
43	Jiumian9	2009	the Northwest Inland Region 甘肃	ICR-CAAS 27×Zhong 65110×Xinluzao7
44	Heishanmian1	1974	the Liaohe River Basin	Jinmian1-derived Lines
45	Liaomian5	1977	the Liaohe River Basin	614007(Liaomian1×Zhong 1470)×ICR-CAAS3
46	Liaomian6	1981	the Liaohe River Basin	[Liaomian3×5998(F ₅)]×[Qiu623×Shuangqing(F ₄)]
47	Liaomian7	1983	the Liaohe River Basin	2034+Xinlu209+Keke4104+Maste 111A+Daizimian16+64-15
48	Liaomian9	1985	the Liaohe River Basin	Liao661×Hei68-35
49	Liaomian10	1990	the Liaohe River Basin	Shan 3215-derived Lines
50	jinmian3	1991	the Liaohe River Basin	Jin496×Sumian1
51	Liaomian12	1994	the Liaohe River Basin	Liao4228×Liao4082
52	Liaomian15	1996	the Liaohe River Basin	Liao 1038 Variant Plant
53	Liaomian16	1999	the Liaohe River Basin	4086×Liaomian6,4696 and 4249
54	Liaomian17	2000	the Liaohe River Basin	78-4084×82-4258
55	Liaomian18	2002	the Liaohe River Basin	87-7109×Liao1038-6
56	Liaomian19	2003	the Liaohe River Basin	Liao205×33B

57	Jinzhong200	1961	YRB×Shanxi	O-д1-derived Lines
58	Lumian1	1976	YRB×Shandong	ICR-CAAS2×1195
59	Sumian1	1988	YRB×Jiangsu	86-1×[1087-2×Daihongdai×DP16×Heishanmian1]
60	Yumian3	1988	YRB×Henan	Shangqiu17×Ke310
61	Yumian5	1988	YRB×Henan	ICR-CAAS10×Heishanmian1×Mianxiang1
62	Lumian10	1990	YRB×Shandong	M-100×ICR-CAAS10
63	Yumian7	1992	YRB×Henan	ICR-CAAS10×Aiganzao×B743F ₃
64	Yumian9	1993	YRB×Henan	Kan 5×ICR-CAAS10F ₁ ×ICR-CAAS14
65	Yumian10	1993	YRB×Henan	Shang40×86-1×Ji366
66	Sumian10	1995	YRB×Jiangsu	Yanmian 48×20-9
67	Sumian11	1997	YRB×Jiangsu	Xiangkan 159×USA CAU'CS-2-81
68	Jinmian23	1997	YRB×Shanxi	Lu 331-derived Lines
69	Jinmian26	1998	YRB×Shanxi	<i>Bt</i> gene into Jinmian 7
70	Jinmian28	1999	YRB×Shanxi	91-57-derived Lines
71	Jinmian34	2002	YRB×Shanxi	VSG×95-1
72	Jinmian36	2003	YRB×Shanxi	Jinmian 19×Yun 148
73	Jinmian44	2005	YRB×Shanxi	<i>CryIAc3</i> gene into Ji 492
74	Lumianyan27	2006	YRB×Shandong	Lu 613×GK12
75	Lumianyan28	2006	YRB×Shandong	Lumian 14×Shiyuan321F ₁ ×5186,Yumian 19,Zhong 12,Zhong 19,Qinyuan 142,Lu8784
76	Kings improved1	1890	the United States	Sugar block cotton
77	Foster cotton6243	-	the United States	Foster cotton
78	Soviet Union91-357	-	the former Soviet Union	Former USSR 61
79	Soviet Union10633	-	the former Soviet Union	Former USSR

1.2 Experimental methods

1.2.1 DNA extraction

Mature leaves collected from the field were flash frozen in liquid nitrogen and ground to a powder. Genomic DNA was extracted from young leaf tissue, by CTAB DNA extraction procedure, as described by Zhang¹⁹ with some

modifications, and the final concentrations were adjusted to 50 ng·μL⁻¹ and stored at -20°C.

1.2.2 PCR amplification and electrophoresis detection

The PCR system consisted of 10 μL reactions containing 1 μL 10× Reaction buffer (including 10 mmol·L⁻¹ MgCl₂), 0.5 μL dNTPs (10 mmol·L⁻¹ of each), 0.4 μL of the forward and reverse primers (10 μmol·L⁻¹), 0.1 μL Taq DNA polymerase (5U·μL⁻¹), 0.5 μL cotton DNA (50 ng·μL⁻¹), and 7.1 μL ddH₂O. The PCR amplification protocol was as follows: pre-denaturation at 95°C for 30 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 59°C for 45 s, and extension at 72 °C for 1 min, with a final extension at 72°C for 3 min. Amplification reactions were stored at 4°C. The PCR products were separated by polyacrylamide gel electrophoresis (PAGE) on 8% gels. Electrophoresis was performed at 200V for 45 min, and the bands were observed by silver staining and photographed.

1.2.3 Band recording and data analysis

DNA fragments amplified with a primer pair that had the same migration position in the PAGE gel were recorded as 1, absence of a band was recorded as 0, bands that were blurred or had a deletion were recorded as 999, and the [0, 1] binary data matrix was constructed. The polymorphism information content of the SSR primers was calculated as ; genotypic diversity was calculated as ; the number of effective alleles per locus was , where P_i represents the gene frequency of the i th allelic variation at a certain locus. Genetic analysis of the 79 cotton accessions was performed using NTSYS-pc2.1 software. The Jaccard similarity coefficient was found using the Qualitative program in Similarity for the original [0,1] binary data matrix obtained from the EST-SSR markers. Based on the genetic similarity coefficient, the UPGMA (unweighted pair group method with arithmetic mean) algorithm in the SAHN program was used for cluster analysis, and the phenogram was generated using the Treeplot module under Graphics.

2. Results And Analysis

2.1 Selection of SSR primers and polymorphisms in the amplified products

A total of 126 pairs of candidate SSR primers were selected by using 8 samples of DNA from ICR-CAAS14 and ‘Xinluzao6’ as templates (Fig. 1), and 71 pairs of SSR primers that gave good polymorphisms, clear amplified bands, and excellent stability were chosen. Except for the unknown chromosome information for 17 pairs of primers, the other primers covered all cotton chromosomes except for Chr. 04, Chr. 08, Chr. 16, and Chr. 26.

A total of 142 effective allelic variants were detected by amplifying DNA from the 79 accessions with the selected 71 pairs of SSR primers. The average number of alleles detected for each SSR primer pair was 2.01, with a range of 1-6. The effective allele numbers ranged from 1.2256 to 10.4502 with an average of 3.4379. The polymorphism information content (PIC) ranged from 0.1841 to 0.9043, with an average of 0.6494. The PIC of the primer pair MON_CGR5565 had the highest value of 0.9043, and the primer pair NAU4044 had the second highest PIC value of 0.8822, whereas the primer pair NAU3181 had the lowest PIC of 0.1841. Genetic diversity (H') ranged from 1.2256 to 10.4502 (Table 2).

Table 2. SSR marker loci, chromosomal locations, and SSR-PCR polymorphism data

SN	SRR locus	Chromosome	PIC	Genetic diversity (H')	Effective Number of Alleles (Ne)
1	NAU4073	Chr.01	0.8168	5.7030	5.4582
2	NAU2457	Chr.01	0.2577	1.5311	1.3471
3	NAU2083	Chr.01	0.4356	1.8725	1.7717
4	NAU4044	Chr.01	0.8822	9.1008	8.4884
5	MON_COT064	Chr.02	0.4980	1.9960	1.9920
6	NAU1190	Chr.03	0.7956	5.3171	4.8933
7	MON_CGR6528	Chr.03	0.7423	3.9381	3.8800
8	MON_CGR6683	Chr.03	0.4576	1.9158	1.8437
9	NAU1269	Chr.05	0.7314	3.8504	3.7234
10	MON_CGR5732	Chr.05	0.7307	3.8447	3.7138
11	NAU1225	Chr.05	0.7307	3.8447	3.7138
12	MON_DC40122	Chr.05	0.6884	3.5157	3.2096
13	NAU1221	Chr.05	0.7307	3.8447	3.7138
14	MON_CGR5651	Chr.06	0.6811	3.4515	3.1354
15	MON_DPL0702	Chr.06	0.7349	3.8787	3.7721
16	MON_COT002	Chr.06	0.7266	3.8116	3.6572
17	BNL1694	Chr.07	0.7442	3.9534	3.9092
18	MUSS095	Chr.07	0.7431	3.9447	3.8921
19	MON_DC30218	Chr.07	0.5000	2.0000	2.0000
20	NAU3859	Chr.09	0.4980	1.9960	1.9920
21	DPL0431	Chr.10	0.6873	3.4966	3.1981
22	NAU3784	Chr.11	0.7071	3.6561	3.4141
23	MON_CER0098	Chr.11	0.7250	3.8002	3.6363
24	NAU3563	Chr.11	0.8579	7.4448	7.0361
25	NAU2671	Chr.12	0.6978	3.5853	3.3095
26	MON_DPL0491	Chr.12	0.2397	1.4972	1.3153
27	NAU3991	Chr.13	0.6801	3.4455	3.1264
28	MON_COT009	Chr.13	0.6886	3.5149	3.2116
29	BNL1421	Chr.13	0.7107	3.6867	3.4567

30	NAU3308	Chr.14	0.6721	3.3728	3.0500
31	GH304	Chr.15	0.7085	3.6704	3.4305
32	MUSS440	Chr.15	0.8784	8.9176	8.2218
33	NAU2343	Chr.15	0.7354	3.8824	3.7790
34	BNL2646	Chr.15	0.6360	3.1112	2.7475
35	NAU2742	Chr.17	0.6635	3.3170	2.9714
36	MON_DPL0308	Chr.18	0.5896	2.7536	2.4365
37	NAU3011	Chr.18	0.4980	1.9960	1.9920
38	NAU5262	Chr.18	0.7407	3.9252	3.8560
39	MON_CGR6151	Chr.19	0.4768	1.9539	1.9115
40	NAU1187	Chr.19	0.7307	3.8447	3.7138
41	NAU1042	Chr.19	0.7307	3.8447	3.7138
42	MON_CGR5590	Chr.19	0.7193	3.7542	3.5631
43	TMB1791	Chr.19	0.7387	3.9101	3.8277
44	MON_CGR6439	Chr.20	0.2604	1.5362	1.3520
45	DPL0442	Chr.20	0.7317	3.8542	3.7271
46	MON_SHIN1421	Chr.20	0.7418	3.9344	3.8728
47	MON_CGR5565	Chr.20	0.9043	11.0904	10.4502
48	BNL1551	Chr.21	0.7006	3.6046	3.3401
49	GH222	Chr.22	0.8094	5.5713	5.2460
50	MON_CGR6410	Chr.22	0.7378	3.9022	3.8136
51	CIR253	Chr.22	0.6158	2.9431	2.6031
52	MUSS139	Chr.23	0.7426	3.9408	3.8848
53	MON_CGR5202	Chr.24	0.3230	1.6552	1.4772
54	MON_CGR6932	Chr.25	0.7423	3.9381	3.8800
55	CRI151		0.7312	3.8485	3.7200
56	MON_CGR6389		0.7430	3.9442	3.8913
57	NAU3181		0.1841	1.3919	1.2256
58	MON_C2-0118		0.7397	3.9179	3.8418
59	CRI002		0.6296	3.0356	2.7000
60	MUCS375		0.3519	1.7103	1.5429

61	MON_CGR6784	0.6384	3.1230	2.7658
62	CIR096	0.7210	3.7659	3.5837
63	NAU3254	0.8289	6.4664	5.8436
64	MON_DPL0133	0.5479	2.4396	2.2119
65	MON_CER0168	0.7397	3.9179	3.8421
66	MUSB0175	0.4734	1.9470	1.8989
67	MON_DPL0906	0.4999	1.9998	1.9997
68	GH111	0.4734	1.9470	1.8989
69	GH112	0.4647	1.9297	1.8680
70	MON_DC40266	0.7037	3.6271	3.3744
71	MON_DC40286	0.6888	3.5120	3.2131

2.2 DNA fingerprinting analysis of the 79 cotton accessions

Fingerprinting analysis of the 79 early-maturing upland cotton accessions was performed using 71 pairs of SSR primers. We found that nine accessions had characteristic bands for which only one primer pair was needed to distinguish each accession from the others. Among them, ICR-CAAS64 had two characteristic primers, 'Xinluzao20', 'Xinluzao25', 'Jiumian9', 'Liaomian5', 'Liaomian17', 'Liaomian19', 'Lumianyan28', and 'Jinmian23' each had one characteristic primer (Table 3). The primer pair NAU4044 was able to uniquely identify four varieties including 'Xinluzao25', 'Jiumian9', 'Lumianyan28', and 'Liaomian 5'. Primer pair NAU3254 could distinguish three varieties, 'Xinluzao20', 'Liaomian17', and 'Liaomian19'. These results indicated that these two primer pairs had abundant polymorphism, strong discrimination power, and numerous characteristic bands, and could be used as preferred markers in the identification of fingerprints.

Table 3. Cotton accessions identified with specific SSR primer pairs

Cultivar	Specific primer	Cultivar	Specific primer
ICR-CAAS64	NAU1190,MUSS440	Xinluzao20	NAU3254
Liaomian17	NAU3254	Liaomian19	NAU3254
Xinluzao25	NAU4044	Jiumian9	NAU4044
Liaomian5	NAU4044	Lumianyan28	NAU4044
Jinmian23	NAU4073		

A total of 55 of the 79 cotton accessions could be identified by three pairs of primers, NAU4044, MUSS440, and MON_CGR5565, which were selected from the 71 pairs of core primers with high PIC values, strong discriminative power, clear bands on the gels, and high reproducibility. Seventy-two varieties could be identified by adding another primer pair, GH222. By adding primer pairs NAU1190 and BNL1694, all 79 of the early-maturing upland cotton varieties could be completely distinguished from one another (Table 4).

Table 4. Fingerprinting data for the 79 early-maturing upland cotton accessions

SN	Cultivar	NAU 4044	MUSS 440	MON_CGR 5565	GH 222	NAU 1190	BNL 1694
1	ICR-CAAS10	10001	00101	001100	001	101	10
2	ICR-CAAS14	10001	11101	001100	010	101	01
3	ICR-CAAS16	10001	11011	001100	001	101	01
4	ICR-CAAS18	10010	00101	001100	001	011	10
5	ICR-CAAS24	00101	00101	101101	101	101	11
6	ICR-CAAS20	10010	00101	001100	100	011	10
7	ICR-CAAS26	10101	00101	110001	001	011	01
8	ICR-CAAS27	10001	11101	001100	110	111	10
9	ICR-CAAS35	11000	11000	010001	001	101	10
10	ICR-CAAS36	10001	11101	001100	101	101	10
11	ICR-CAAS37	00100	11000	001010	010	101	01
12	ICR-CAAS42	00100	00101	100001	100	011	10
13	ICR-CAAS50	10001	11000	100001	100	101	10
14	ICR-CAAS58	10100	00101	100001	100	011	10
15	ICR-CAAS64	00100	00000	100001	100	000	10
16	Xinluzao1	11000	00101	100001	100	101	01
17	Xinluzao3	10101	00101	101101	010	101	01
18	Xinluzao4	10001	00011	100001	100	010	01
19	Xinluzao6	10010	11000	001100	001	101	01
20	Xinluzao7	10001	11000	100001	010	111	01
21	Xinluzao9	10010	00101	100001	001	101	01
22	Xinluzao10	01010	11101	100001	001	011	01
23	Xinluzao11	10101	11101	001110	001	111	11
24	Xinluzao12	11101	00101	001110	010	101	11
25	Jiumian2	10100	00101	001100	101	101	01
26	Xinluzao13	10001	11000	010001	001	101	10
27	Xinluzao20	11101	01111	001100	110	101	11
28	Xinluzao22	11000	00101	001010	100	101	10
29	Xinluzao23	10100	00101	111111	001	101	01

30	Xinluzao25	11011	11011	001110	101	101	01
31	Xinluzao26	00011	11011	001110	001	110	11
32	Xinluzao27	10001	11000	001100	001	011	10
33	Xinluzao30	10100	00101	001100	100	101	01
34	Xinluzao31	10010	00101	001100	001	101	01
35	Xinluzao32	01010	11000	100001	001	011	10
36	Xinluzao34	01000	00011	100001	001	101	10
37	Xinluzao35	00101	00101	111111	001	111	11
38	Xinluzao36	10010	11000	100001	010	011	01
39	Xinluzao37	00101	01111	001110	001	101	01
40	Jiumian3	00100	00101	001100	101	101	01
41	Xinluzao39	01000	00101	010001	001	011	10
42	Jiumian8	00100	11000	001100	001	101	01
43	Jiumian9	10011	00101	101101	100	101	01
44	Heishanmian1	10001	11000	001010	001	011	10
45	Liaomian5	00001	00101	100001	010	011	01
46	Liaomian6	00100	00101	001010	010	101	01
47	Liaomian7	00100	00101	001100	100	101	01
48	Liaomian9	10101	00101	001100	011	101	11
49	Liaomian10	11101	00101	001100	010	101	10
50	Liaojinmian3	10100	11000	001010	001	101	01
51	Liaomian12	00100	00101	001100	011	101	01
52	Liaomian15	10100	00011	001100	001	-	10
53	Liaomian16	10100	00101	-	010	101	10
54	Liaomian17	00100	01111	001100	001	101	01
55	Liaomian18	10100	00011	001100	010	100	10
56	Liaomian19	00100	01111	101101	010	010	10
57	Jinzhong200	10001	00101	100001	010	101	10
58	Lumian1	10001	01111	111111	001	110	10
59	Sumian1	00100	00011	001010	010	101	10
60	Yumian3	10100	00101	111111	011	011	11

61	Yumian5	11000	00101	001100	001	101	10
62	Lumian10	10010	00101	110001	001	101	01
63	Yumian7	10100	00101	001010	100	101	10
64	Yumian9	10001	00101	001100	001	101	01
65	Yumian10	11100	00101	010001	001	011	01
66	Sumian10	10101	00011	001100	011	010	01
67	Sumian11	11100	00101	110001	001	101	11
68	Jinmian23	11111	11101	111111	101	101	10
69	Jinmian26	11111	11101	101101	101	101	01
70	Jinmian28	10100	11101	101101	011	101	01
71	Jinmian34	10100	00101	100001	001	101	01
72	Jinmian36	11000	00011	010001	010	010	01
73	Jinmian44	00100	00011	100001	001	010	01
74	Lumianyan27	10100	00011	-	001	010	01
75	Lumianyan28	01100	01111	110001	010	010	01
76	Kings improved1	10001	00101	001100	001	011	10
77	Foster cotton6243	10100	00101	001100	001	101	10
78	Soviet Union91-357	00011	00101	001100	011	110	10
79	Soviet Union10633	10100	00011	001100	001	100	01

2.3 Genetic diversity analysis

Similarity coefficients between varieties were calculated using NTSYS-pc 2.11 and Microsoft Excel software. The results showed that the genetic similarity coefficients among the 79 early-maturing upland cotton accessions ranged from 0.3310 ('Jinmian36' and 'Xinluzao25') to 0.8705 ('Liaomian15' and 'Liaomian18'), with an average of 0.5861, indicating that 'Jinmian36' and 'Xinluzao25' have the highest genetic diversity, while 'Liaomian15' and 'Liaomian18' have the lowest. The similarity coefficients between varieties that were <0.4 (large genetic difference) accounted for 1.1%, 11.9% at 0.4-0.5, 44.1% at 0.5-0.6, 35.2% at 0.6-0.7, and 7.1% at 0.7-0.8. The similarity coefficients >0.8 accounted for 0.5% (Fig. 2). This demonstrates that the genetic relationships are relatively close among the 79 early-maturing upland cotton accessions, but that some genetic diversity is still present.

We performed genetic diversity analysis of the 79 early-maturing upland cotton accessions from six regions that included the China Cotton Institute, YRB (Henan, Shanxi, Shandong, Jiangsu), the Northwest Inland Cotton Region (Xinjiang, Gansu), the Liaohe River Basin, the United States, and the former Soviet Union. By comparison, the accessions from the former Soviet Union had the smallest average genetic similarity coefficients of the six regions, which increased in the order of the YRB cotton area, the United States, the China Cotton Institute, the

Liaohe River Basin Early Maturing Cotton Area, and the Northwest Inland Cotton Area, indicating that there is ample genetic diversity in cotton resources imported from abroad. At the same time, the genetic diversity of accessions from the YRB cotton region is relatively high, which may be related to the complex geographical diversity of the YRB cotton region and the dispersion of breeders in Henan, Shanxi, Shandong, Jiangsu, and other provinces.

We found that the genetic similarity coefficients of accessions from the six regions were between 0.5575 and 0.6143, and the highest similarity coefficients were found between accessions from China and the USA. This indicates that early-maturing upland cotton varieties selected by ICR-CAAS have close genetic relationships to selections from the USA. In general Chinese cotton germplasm is more frequently exchanged for resources from the USA compared with other regions. The lowest genetic similarity coefficient in the early-maturing cotton areas is between YRB and the Liaohe River Basin, with a value of 0.5575, and the second lowest is between YRB and the Northwest Inland Cotton Area, with a value of 0.5636 (Table 5). The underlying reason for this may be that the YRB cotton-growing area has a better climate with warmer conditions, resulting in more varieties of early-maturing upland cotton and larger differences between varieties than the early-maturing cotton areas of the Liaohe River Basin and the Northwest Inland Cotton Area.

Comparisons of the genetic similarity coefficients between domestic and foreign early-maturing upland cotton varieties showed that except for the Northwestern Inland Cotton Area, the genetic similarity coefficients between cotton varieties from the ICR-CAAS, YRB, and the Liaohe River Basin are higher. This suggests that early-maturing upland cotton grown in the ICR-CAAS, YRB, and Liaohe River Basin in the early-maturing cotton area contains more American germplasm. Because of the introduction and utilization of early-maturing upland cotton in China, the majority of early maturity genetic resources came from American gold-colored cotton. The genetic similarity coefficients between accessions from the Northwestern Inland Cotton Region and the former Soviet Union is relatively high. This may be because the Northwest Inland Cotton Region is adjacent to the former Soviet Union, so it is easier to introduce germplasm resources into China from there.

Table 5. Genetic diversity of cotton cultivars from the six cotton-growing regions in China

Region	Genetic similarity coefficient	CAAS	YRB	the Northwest Inland Region	the Liaohe River Basin	the United States	the former Soviet Union
CAAS	Max	0.8058	0.7817	0.8169	0.8451	0.7447	0.6691
	Min	0.3630	0.3643	0.3704	0.3582	0.4789	0.4296
	Mean	0.6032	0.5796	0.5943	0.5824	0.6143	0.5672
YRB	Max		0.7899	0.8028	0.7606	0.7042	0.7042
	Min		0.4085	0.3310	0.3475	0.3521	0.4366
	Mean		0.5802	0.5636	0.5575	0.5911	0.5661
the Northwest Inland Region	Max			0.8239	0.8310	0.7324	0.7254
	Min			0.4225	0.3582	0.4296	0.4296
	Mean			0.6221	0.5936	0.5799	0.5894
the Liaohe River Basin	Max				0.8705	0.7465	0.6812
	Min				0.3944	0.4718	0.4014
	Mean				0.6134	0.5783	0.5684
the United States	Max					0.5845	0.6549
	Min					0.5845	0.5357
	Mean					0.5845	0.6027
the former Soviet Union	Max						0.5286
	Min						0.5286
	Mean						0.5286

The results of our study show that the average genetic similarity coefficient of bred and certified varieties of early-maturing upland cotton varieties from different areas in China showed a low-high-low pattern of variation as determined by genetic diversity analysis. ‘Jinzhong200’, ‘Xinluzao1’, ‘Lumian1’, ‘Heishanmian1’, and ‘Liaomia5’, which were selected prior to the 1980s, had the lowest genetic similarity coefficient of 0.5704. The nine varieties ‘ICR-CAAS10’, ‘ICR-CAAS14’, ‘Xinluzao3’, ‘Liaomian6’, ‘Liaomian7’, ‘Liaomian9’, ‘Sumian1’, ‘Yumian3’, and ‘Yumian5’, which were selected in the 1980s, have the highest average genetic similarity coefficient of 0.6306. The 29 varieties selected in the 1990s, which include ‘ICR-CAAS16’, ‘ICR-CAAS18’, ‘Xinluzao4’, ‘Liaomian10’, ‘Lumian10’, ‘Yumian7’, ‘Sumian10’, and ‘Jinmian23’, have an average genetic similarity coefficient of 0.5993. The average similarity coefficient of 32 varieties including ‘ICR-CAAS42’, ‘ICR-CAAS50’, ‘Xinluzao13’, ‘Jiumian2’, ‘Liaomian17’, ‘Jinmian34’, and ‘Lumianyan27’ selected after 2000 is 0.5791.

The average genetic similarity coefficients of early-maturing upland cotton varieties in China have shown a low-high-low pattern over time (Fig. 3). This may be because before the 1980s, domestic early-maturing upland cotton breeding was mainly carried out by introducing different early-maturing varieties from abroad and systematically using them in breeding. Since the early 1980s, cotton production and the cotton spinning industry

have developed rapidly. Due to economic reform and the opening up of the country, transportation is more convenient, and the exchange of germplasm resources between breeding units has become frequent. In particular, a number of outstanding varieties (lines) such as 'Heishanmian1' and 'ICR-CAAS10' stand out from the competition and are used by other breeders as donor parents. This has resulted in closer genetic relationships between the varieties selected at this time, with higher genetic similarity coefficients and less genetic difference. In the 1990s, the difficulties of domestic distant hybridization were continuously overcome, and breeders consciously chose parental materials with complex genetic backgrounds for cross-breeding, which resulted in a significant reduction in the genetic similarity coefficients of cotton varieties and increased the genetic difference. After 2000, the use of modern breeding technologies (transgenics and molecular marker-assisted breeding) not only accelerated the cotton breeding process, but also broadened the source of available cotton genes, resulting in further reductions in the genetic similarity coefficients among new varieties of early-maturing upland cotton in China²⁰.

Based on the Jaccard similarity coefficient, 79 early-maturing upland cotton varieties were grouped using a hierarchical clustering method (UPGMA) (Fig. 4). The results showed that at a genetic similarity coefficient of 0.87, all 79 early-maturing upland cotton varieties were completely separated. At a similarity coefficient of 0.57, the 79 main varieties could be divided into five categories or classes. Class I contains 42 varieties, including seven varieties from the China Cotton Institute, 20 varieties from the Northwest Inland Cotton Area, nine varieties from the special early-maturing cotton area of the Liaohe River Basin, four varieties from the YRB cotton area, one variety from the US, and one variety from the former Soviet Union. Class II contains 27 varieties, including six varieties from the China Cotton Institute, six varieties from the Northwest Inland Cotton Area, two varieties from the special early-maturing cotton area of the Liaohe River Basin, 11 varieties from the YRB cotton area, and one variety each from the United States and the former Soviet Union. Class III has only varieties, both of which are from the Northwestern Inland Cotton Area; Class IV includes a single variety from YRB; and Class V contains seven varieties, including two from Zhongmian, two varieties from the Liaohe Basin early-maturing cotton area, and three varieties from the YRB cotton area. Among these five classes, most of the selected varieties of cotton grown in China clustered in Class I and Class II, accounting for 46.7% and 40.0% respectively. Most of the varieties from the Northwestern Inland cotton area are in Class I, accounting for 71.4%; most of varieties from the Liaohe Basin are concentrated in Class I, accounting for 69.2%; and most of the cotton varieties from YRB are concentrated in Class II, accounting for 57.9%. This shows that the clustering results reflect certain geographical distribution characteristics, and the genetic differences of the cultivars from the same area are relatively small, which is why they cluster together.

3. Discussion

In recent years, SSR molecular marker technology has been widely used in the genetic diversity and fingerprinting of cotton germplasm resources. For example, Han et al.²¹ used SSR markers to construct fingerprints and analyze the genetic diversity of 27 cotton accessions from 2009 to 2010. Li et al.²² used 20 pairs of SSR primers to construct fingerprints of the BaiMian cotton series. Kuang et al.²³ found a correlation between cultivars and geographical origins by analyzing the genetic diversity in the main cotton varieties in China using SSR markers. These studies were important for the identification and purity detection of cotton varieties in China, as well as understanding the genetic relationships and origins of cotton varieties at the

molecular level, which provided a theoretical basis for rational selection of hybrid parents and the breeding of new varieties of cotton.

However, there are few reports on the use of SSR molecular markers to study early-maturing upland cotton in China. In our study, we constructed DNA fingerprints of 79 early-maturing upland cotton accessions using 73 SSR markers and analyzed the molecular data to determine genetic similarities. We found that 72 main varieties can be divided into five categories (Classes I, II, III, IV, and V) with a genetic similarity coefficient of 0.57, and the clustering results for the 79 early-maturing upland cotton accessions were consistent with the pedigree (Fig. 5). For example, 'Liaomian15' and 'Liaomian18' were first clustered together, traced back to their pedigree sources, and were both found to contain 'Liao 1038' in their ancestry. 'Xinluzao6', 'Xinluzao9', 'Xinluzao27', 'Xinluzao31', and 'Xinluzao39' are grouped together in Class I, and their pedigree sources show that they all are descended from 'Bell Snow'. 'Xinluzao6', 'Xinluzao9', and 'Xinluzao27' clustered into a group with a genetic similarity coefficient of 0.87. We also identified several cases of incomplete matching. For example, 'Jiu Mian 2' is in the 'ICR-CAAS16' lineage; 'Jinmian3' is in the 'Sumian1' lineage; seven varieties including 'ICR-CAAS16', 'Zhongmian24', 'ICR-CAAS26', 'Yumian5', 'Yumian7', 'Yumian9', and 'Lumian10' are in the 'Zhongmian10' lineage; three varieties including 'ICR-CAAS10', 'ICR-CAAS18', and 'Sumian 1' are in the 'Heishan Mian1' lineage; and three varieties including 'Heishan1', 'Jinzhong200', and 'Jinzi 1' descend from the gold-line cotton pedigree. The clustering results indicate that seven varieties including 'ICR-CAAS10', 'Jiu Mian2', 'ICR-CAAS16', 'Yumian5', 'Yumian9', 'Jinmian3', and 'Lumian10' group together in Class I. Eight varieties including 'Sumian1', 'Zhongmian24', 'Zhongmian26', 'Yumian7', 'ICR-CAAS18', 'Heishan Mian1', 'Jinzhong200', and 'Kings improved1' are in Class II.

In addition, the genetic relationship of 'ICR-CAAS37' and 'Liaomian6' seem to be relatively distant but can be grouped together. However, the genetic relationship of 'Liaomian6' and 'Liaomian16' seem to be relatively close but do not cluster together. This suggests that the classification is not entirely dependent on pedigree, but is also influenced by the selection method, breeding process, and target traits. Pedigree analysis can only reflect the relative genetic information between the varieties. Molecular marker analysis using marker loci distributed over the whole genome can more accurately reflect the genetic differences between varieties²⁴⁻²⁵.

Declarations

Author Contributions: H.D.C designed the experiments. Z.C.K, H.D.C, L.S.G, C.S.X, and J.Y.Z conceived the experiments and analyzed the results. Z.C.K, H.D.C, C.S.X, L.S.G, J.Y.Z carried out all computational analyses. Z.C.K, H.D.C, C.S.X, L.S.G, J.Y.Z, W.W, B.H.W, H.H, Y.J.L and Y.Q.L participated in part of experiments. H.D.C, Z.C.K, and H.H drafted the manuscript. H.H proofread and H.D.C, B.H.W revised the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

A silver stained gel showing primer screening for polymorphic SSRs

Figure 2

Similarity coefficients for the 79 early-maturing upland cotton accessions

Figure 3

The genetic similarity coefficients of early-maturing Upland cotton varieties developed over the past four decades

Figure 4

The genetic lineages of the 72 early-maturing upland cotton varieties based on UPGMA clustering

Figure 5

The pedigrees of 15 upland cotton varieties